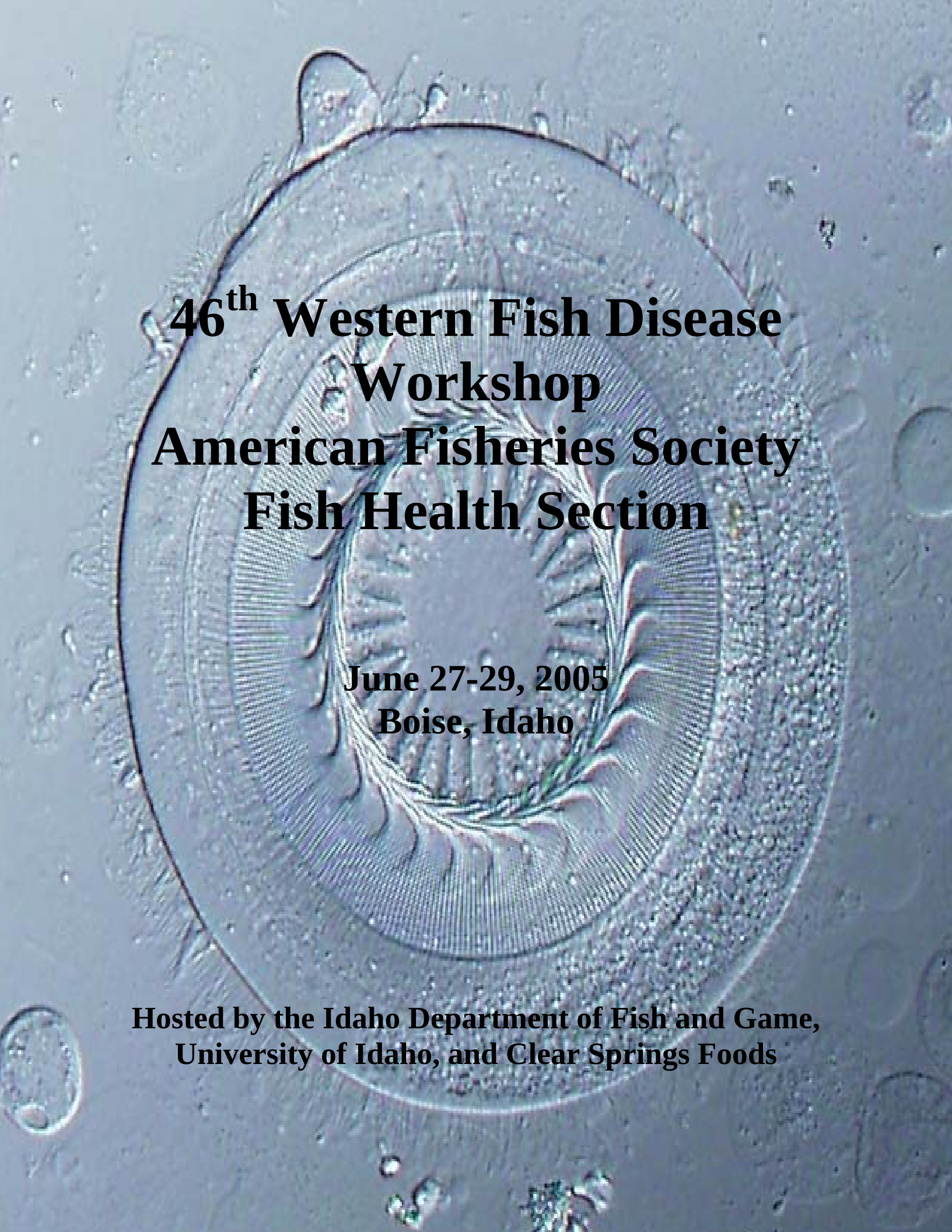

46th Western Fish Disease Workshop
American Fisheries Society Fish Health Section

June 27-29, 2005
Boise, Idaho

Hosted by the Idaho Department of Fish and Game, University of Idaho, and Clear Springs Foods

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46TH WESTERN FISH DISEASE WORKSHOP – BOISE, IDAHO
PROGRAM AT A GLANCE

Sunday, June 26th, 2005	
Registration	7:00 – 9:00 PM
Monday, June 27th, 2005	
Registration	7:00 – 8:30 AM
	7:00 – 9:00 PM
Continuing Education Class (Risk assessment)	
Morning session	8:30 – 11:45 AM
Break	10:00 – 10:15 AM
Lunch	11:45 – 12:45 AM
Afternoon session	12:45 – 4:30 PM
Split in 3 groups	1:15 PM
Reconvene	2:30 PM
Break	3:00 – 3:15 PM
End	4:30 PM
Tuesday, June 28th, 2005	
Registration	7:00 – 8:50 AM
Introductory Remarks	8:50 – 9:00 AM
Session 1	9:00 – 10:20 AM
Coffee break	10:20 – 10:40 AM
Session 1 (continued)	10:40 – 12:00 PM
Lunch	12:00 – 1:30 PM
Session 2	1:30 – 2:50 PM
Afternoon break	2:50 – 3:10 PM
Session 2 (continued)	3:10 – 4:30 PM
SOCIAL AND POSTER SESSION	5:30 - ?
Wednesday, June 29th, 2005	
Session 3	9:00 – 10:10 AM
Coffee break	10:10 – 10:30 AM
Session 3 (continued)	10:30 – 12:00 PM
Lunch	12:00 – 1:30 PM
Session 4	1:30 – 2:50 PM
Afternoon break	2:50 – 3:10 PM
Session 4 (continued)	3:10 – 3:50 PM
ADJOURN	4:00 PM

46th Western Fish Disease Workshop
AFS Fish Health Section
Doubletree Hotel, Boise Riverside
Boise, Idaho
June 27-29th

Monday , June 27

8:30 am	**Continuing Education Class** APPLYING RISK ASSESSMENT PRINCIPLES TO FISH HEALTH SITUATIONS

Tuesday, June 28

Session 1 – **Warren Groberg, moderator**

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9:20	Bill Batts	IHN VIRAL GENOGROUP-SPECIFIC PATHOGENICITY IN SOCKEYE SALMON (*Oncorhynchus nerka*)	2
9:40	Maureen Purcell	IMMUNOLOGICAL RESPONSE OF REDFISH LAKE SOCKEYE SALMON TO THE U AND M GENOGROUPS OF INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS	3
10:00	Garth Traxler	EARLY DETECTION OF INFECTIOUS HEMATOPOEITIC NECROSIS (IHN) VIRUS IN ATLANTIC SALMON AND SUSCEPTIBILITY OF PACIFIC SALMON SMOLTS TO IHNV	4
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10:40	Bill Batts	GENETIC ANALYSIS OF COMPLETE GENES AND GENOMES OF NORTH AMERICAN INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS	5
11:00	Maureen Purcell	MICROARRAY ANALYSIS OF HOMOZYGOUS RAINBOW TROUT FOLLOWING DNA VACCINATION AGAINST IHNV	6
11:20	Eric Landis	TRANSCRIPTIONAL REGULATION OF TAPASIN, A VIRALLY INDUCED MHC CLASS I PATHWAY MEMBER	7
11:40	Scott LaPatra	INACTIVATED IHN VIRUS ELICITS AN EARLY ANTIVIRAL RESPONSE	8
12:00		**LUNCH**	

<u>Session 2</u> – Christine Moffitt, moderator

1:30	Richard Stocking	ECOLOGY OF THE SALMONID PARASITE *Ceratomyxa shasta* IN THE KLAMATH RIVER BASIN	9
1:50	Sascha Hallet	APPLICATION OF A REAL-TIME PCR ASSAY TO DETECT THE MYXOZOAN PARASITE *CERATOMYXA SHASTA* IN ENVIRONMENTAL WATER SAMPLES	10
2:10	Leyla Arsan	FISH AT-RISK? A RISK ASSESMENT FOR THE INTRODUCTION AND ESTABLISHMENT OF *Myxobolus cerebralis* INTO THE WILLAMETTE RIVER, OR AND THE STATE OF ALASKA	11
2:30	Robert DuBey	SUSCEPTIBILITY OF RIO GRANDE CUTTHROAT TROUT (*Oncorhynchus clarki virginalis*) TO EXPERIMENTALLY INDUCED INFECTION WITH *Myxobolus cerebralis*	12
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3:30	Nicole Lindstrom	DEVELOPMENT OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR DETECTION OF *Flavobacterium psychrophilum*	14
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6.	Virginia Watral	BIOCHEMICAL AND MOLECULAR TECHNIQUES FOR STRAIN TYPING OF *Mycobacterium marinum*	22

Wednesday, June 29

Session 3 – Garth Traxler, moderator

Session 4 – Mike Kent, moderator

3:30	Grace Karreman	BCSFA FISH HEALTH DATABASE – RETROSPECTIVE OVERVIEW	35

4:00 **ADJOURN**

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2. DEVELOPMENT AND DISORDERS OF OPAKAPAKA (*PRISTIPOMOIDES FILAMENTOSUS*) LARVAE IN CULTURE
   Briana Keafer* and Michael Kent

3. DIFFERENTIAL VIRAL SUSCEPTIBILITY OF SACRAMENTO RIVER FALL-RUN CHINOOK SALMON (*Oncorhynchus tshawytscha*) FRY AND ALEVIN TO SIMULATED ADULT SALMON SHEDDING OF INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS IN A LOTIC ENVIRONMENT
   J. Scott Foott* , Rick Harmon & Ron Stone

4. FISH AT-RISK? A RISK ASSESMENT FOR THE INTRODUCTION AND ESTABLISHMENT OF Myxobolus cerebralis INTO THE WILLAMETTE RIVER, OR AND THE STATE OF ALASKA
   Leyla Arsan* Sascha Hallet and Jerri Bartholomew

5. THE LIFE CYCLE OF *Parvicapsula minibicornis*, A MYXOZOAN PARASITE OF SALMONIDS, INVOLVES THE FRESHWATER POLYCHAETE Manayunkia speciosa
   J. L. Bartholomew*, S. D. Atkinson, S. L. Hallett and C. Zielinski

6. SUSCEPTIBILITY OF RIO GRANDE CUTTHROAT TROUT (Oncorhynchus clarki virginalis) TO EXPERIMENTALLY INDUCED INFECTION WITH Myxobolus cerebralis
   Robert DuBey*, Colleen Caldwell, and William R. Gould

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   S. L. Hallett* and J. L. Bartholomew

8. TRANSCRIPTIONAL REGULATION OF TAPASIN, A VIRALLY INDUCED MHC CLASS I PATHWAY MEMBER
   Eric D. Landis* and John D. Hansen

9. DOES THE SALMON LOUSE *Lepeophtheirus salmonis krøyer* OCCUR ON THREESPINE STICKLEBACK (*Gasterosteus aculeatus* L.) IN COASTAL BRITISH COLUMBIA?
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10. DEVELOPMENT OF MARKERS FOR T LYMPHOCYTES IN SALMONID FISH: GENERATION OF SPECIFIC MONOCLONAL ANTIBODIES AGAINST RAINBOW TROUT LCK
    Kerry J. Laing* and John D. Hansen

11. INACTIVATED IHN VIRUS ELICITS AN EARLY ANTIVIRAL RESPONSE
    S.E. LaPatra*, S.C. Clouthier, W.D. Shewmaker, M.L. Higgins, A.E. Weighall, and E.D. Anderson

12. COMBINING SUPPRESSION SUBTRACTIVE HYBRIDIZATION AND MICROARRAYS TO MAP THE INTRA-SPECIFIC PHYLOGENY OF *Flavobacterium psychrophilum*
    Marilyn Soule*, Stacey LaFrentz, Kenneth Cain, Scott LaPatra, and Douglas R. Call

13. MICROARRAY ANALYSIS OF HOMOZYGOUS RAINBOW TROUT FOLLOWING DNA VACCINATION AGAINST IHNV
    Maureen K. Purcell*, Krista M. Nichols, Linda K. Park, Gael Kurath, Gary H. Thorgaard, Paul Wheeler and James R. Winton

14. ECOLOGY OF THE SALMONID PARASITE *Ceratomyxa shasta* IN THE KLAMATH RIVER BASIN
    R.W.Stocking*, R.A. Holt, S.J. Foott, and J.L.Bartholomew

15. SOMETHING OLD, SOMETHING NEW: MYCOBACTERIOSIS IN SALMON AND ZEBRAFISH
    C. M. Whipps* and M. L. Kent

16. EARLY DETECTION OF INFECTIOUS HEMATOPOEITIC NECROSIS (IHN) VIRUS IN ATLANTIC SALMON AND SUSCEPTIBILITY OF PACIFIC SALMON SMOLTS TO IHNV
    Garth Traxler*, Jon Richard and Kerry Bate

17. BREAKING THE CYCLE: USING COMBINATIONS OF ANTIBIOTICS AND VACCINATION TO PREVENT AND TREAT BACTERIAL KIDNEY DISEASE
    Linda D. Rhodes*, Lee W. Harrell, Cindra K. Rathbone, Rebecca K. Deinhard, Mark S. Strom, William T. Fairgrieve, and Alison M. Coady

18. IHN VIRAL GENOGROUP-SPECIFIC PATHOGENICITY IN SOCKEYE SALMON (*Oncorhynchus nerka*)
    Bill Batts*, Kyle Garver, Keith Johnson, Scott LaPatra, and Gael Kurath

19. EVALUATION OF LETHAL AND NON-LETHAL SAMPLING FOR THE DETECTION OF WSIV INFECTION IN KOOTENAI RIVER WHITE STURGEON (*Acipenser transmontanus*)
    John D. Drennan*, Scott E. LaPatra, Corie A. Samson, Sue Ireland, and Kenneth D. Cain

20. IMMUNOLOGICAL RESPONSE OF REDFISH LAKE SOCKEYE SALMON TO THE U AND M GENOGROUPS OF INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS
    Maureen K. Purcell*, Kyle A. Garver and Gael Kurath

21. DEVELOPMENT OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR DETECTION OF *Flavobacterium psychrophilum*
    Nicole M. Lindstrom*, Stacey A. LaFrentz, Douglas R. Call, and Kenneth D. Cain

22. SURVIVAL OF NEW ZEALAND MUDSNAILS IN RAINBOW TROUT (ONCORHYNCHUS MYKISS) GASTROINTESTINAL TRACTS
    R. Louise Bruce* and Dr. Christine M. Moffitt

23. RISK CHARACTERIZATION OF ERYTHROMYCIN EFFLUENTS FROM SALMON HATCHERIES
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24. MICROBIAL PROFILES FROM RAINBOW TROUT INTESTINES, NEW ZEALAND MUDSNAILS, AND HATCHERY INFLOW AND OUTFLOW AT HAGERMAN STATE FISH HATCHERY, IDAHO
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25. GENETIC ANALYSIS OF COMPLETE GENES AND GENOMES OF NORTH AMERICAN INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS
    Bill Batts*, Gael Kurath, and Jim Winton

26. DISSEMINATED NEOPLASMS OF EASTERN PACIFIC MUSSELS, *Mytilus trossulus*
    James D. Moore*, Elizabeth Vu, Ronald P. Hedrick

27. EVALUATION OF MACROPHAGE AGGREGATES IN SALMONID FISHES
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28. CHANGES IN HEMATOLOGY IN ATLANTIC SALMON AFTER EXPERIMENTAL CHALLENGE WITH IHN VIRUS: PRELIMINARY RESULTS
    Grace Karreman* and Garth Traxler

29. BCSFA FISH HEALTH DATABASE – RETROSPECTIVE OVERVIEW
    Grace Karreman*

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1. EFFECTS OF TEMPERATURE AND DESICCATION ON *Ceratomyxa shasta* ACTINOSPORE PRODUCTION AND RELEASE FROM THE POLYCHAETE HOST *Manayunkia Speciosa*
   S. J. Bjork*, S. L. Hallett, and J. L. Bartholomew

2. PREVALENCE OF *Renibacterium salmoninarum* INFECTION AMONG JUVENILE CHINOOK SALMON IN NORTH PUGET SOUND AND IMPLICATIONS FOR DISEASE INTERACTIONS
   Shelly Nance, Colleen Durkin, Casimir Rice, and Linda Rhodes*

3. DETECTION OF *Myxidium* spp. IN PET SHOP GOLDFISH
   C. M. Zielinski*, S. D. Atkinson, and J. L. Bartholomew

4. MULTIFARIOUS MYXOZOANS: SPORE HUNTING IN OREGON
   S. D. Atkinson*, S. L. Hallett and J. L. Bartholomew

5. CHARACTERIZATION OF THE IDAHO NEUROTROPIC *Myxobolus*, AND IT'S DISTRIBUTION IN IDAHO
   Carla Hogge* and Keith Johnson WATERS

6. BIOCHEMICAL AND MOLECULAR TECHNIQUES FOR STRAIN TYPING OF *Mycobacterium marinum*
   Virginia Watral*, Christopher Whipps, Vaughn Ostland, Frank Austin, and Michael Kent

ORAL PRESENTATIONS

DIFFERENTIAL VIRAL SUSCEPTIBILITY OF SACRAMENTO RIVER FALL-RUN CHINOOK SALMON (*Oncorhynchus tshawytscha*) FRY AND ALEVIN TO SIMULATED ADULT SALMON SHEDDING OF INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS IN A LOTIC ENVIRONMENT.

J. Scott Foott* , Rick Harmon & Ron Stone

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Chinook salmon alevin and fry were exposed to IHNV by either 30 min static bath or 4 L / s flow-through challenges in 2 experiments. In the first experiment, alevins were challenged by 10^3 to 10^6 pfu / mL of virus. No mortality due to viral infection occurred over 21 days. Virus was only isolated from alevins challenged by static bath. Infected cells, within both the thymus and epidermis, were detected by immunohistochemistry (IHC) from alevins collected 24 hrs post-exposure. Water samples collected 20 s post-inoculation (PI) contained $> 10^3$ pfu / mL .

In the second experiment, alevin and fry (0.5g) were challenged as in experiment 1 by a virus suspension of 10^8 pfu / mL. Virus concentration in the 20 s PI water samples ranged from 8×10^2 to 6.4×10^3 pfu / mL . Mortality was < 2% in alevins exposed by flow-through challenges with ≤ 10% virus detection. Fry incurred 6 – 16% mortality from similar flow-through challenges with > 50% virus detection in 20 day post-exposure samples. Static bath challenges (10^8 pfu / mL) resulted in 34% mortality in alevins and 100% virus isolation. Systemic infections were only observed by IHC in exposed fry. Unlike fry, Sacramento River Fall-run Chinook alevins were largely resistant to disease by IHNV. It would appear that external infections did not always progress into systemic infections. This data suggests that movement of IHNV infected adult salmon into reaches of Battle Creek, where Spring-run Chinook (threatened status) alevins may be present, does not pose a significant health risk to the alevins.

IHN VIRAL GENOGROUP-SPECIFIC PATHOGENICITY IN SOCKEYE SALMON (*Oncorhynchus nerka*)

Bill Batts[1], Kyle Garver[1,2], Keith Johnson[3], Scott LaPatra[4], and Gael Kurath[1,2]

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[2]Department of Pathobiology, University of Washington, Seattle, WA 98195
[3]Eagle Fish Health Laboratory, IDFG, 1800 Trout Road, Eagle, ID 83616
[4]Clear Springs Foods Inc., PO Box 712, Buhl, ID 83316

Infectious hematopoietic necrosis virus (IHNV) is the most important viral pathogen affecting wild and cultured salmonid fish in North America. The virus is currently endemic from Alaska to California and inland to Idaho. Within this geographic area, IHNV isolates phylogenetically group into three major genogroups designated U, M, and L. In this study, seven IHNV isolates (3 U, 3 M, and 1 L) were tested for pathogenicity in Redfish Lake sockeye salmon. Fish challenged by either waterborne immersion or intraperitoneal (IP) injection of IHNV were highly susceptible to U genogroup virus infection but were refractory to M genogroup virus infection, while L genogroup virus showed intermediate pathogenicity. Moreover an M genogroup virus immersion challenge did not confer protection against a subsequent U genogroup virus challenge. However a high dose IP injection challenge with an M genogroup isolate did protect fish against a subsequent U genogroup challenge, suggesting that the viral challenge dose and/or infection route is important in eliciting a protective immune response against an M genogroup virus in sockeye salmon. To gain insight into the molecular basis of the differences in pathogenicity between the different virus genogroups complete glycoprotein gene sequencing was performed.

IMMUNOLOGICAL RESPONSE OF REDFISH LAKE SOCKEYE SALMON TO THE U AND M GENOGROUPS OF INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS

Maureen K. Purcell[1]*, Kyle A. Garver[2] and Gael Kurath[3]

[1]School of Aquatic and Fishery Sciences, University of Washington, Seattle WA
[2]Department of Pathobiology, University of Washington, Seattle WA
[3]Western Fisheries Research Center, USGS, Seattle WA

Molecular characterization of infectious hematopoietic necrosis virus (IHNV) in the Western United States has established 3 main genogroups (U, M and L). The U genogroup ranges from Alaska through Oregon while the M genogroup is restricted to the Columbia River basin. Ongoing studies in our laboratory have established that the U genogroup is highly pathogenic to Redfish Lake sockeye salmon (*Oncorhynchus nerka*), causing 95-100% cumulative percent mortality (CPM), while the M genogroup is nearly non-pathogenic, with only 0-5% CPM. In this study we sought to address whether an M genogroup virus could replicate in the sockeye host and if it elicited an innate immune response. To that end, we challenged Redfish Lake sockeye salmon by both immersion and injection with either a representative U viral strain (Baker Lake 94) or a representative M strain (220-90), and sampled fish at days 0, 3, 7 and 14. Viral titers showed that fish challenged with both strains were positive for virus on days 3 and 7 but the fish exposed to the M genogroup virus had approximately 2 logs less plaque forming units (PFU) than those exposed to the U genogroup virus. To assess the host innate immune response, Mx-1 gene expression was evaluated in the anterior kidney by quantitative RT-PCR (qRT-PCR). Although both viruses stimulated an Mx response it was significantly lower in M virus challenged fish. The pattern of Mx-1 gene expression was highly associated with viral titer. In ongoing studies, we are further evaluating this relationship by comparing the Mx-1 gene expression to the number of viral genome copies in the anterior kidney using an IHNV negative-sense strand specific qRT-PCR assay. The main conclusions from this study are that the M viral strain did replicate and stimulate an innate immune response in the sockeye salmon, but to significantly lower extents than the U strain. It is possible that mortality is related to a threshold level of virus but the exact mechanisms of differential pathogenicity have not been conclusively determined.

EARLY DETECTION OF INFECTIOUS HEMATOPOEITIC NECROSIS (IHN) VIRUS IN ATLANTIC SALMON AND SUSCEPTIBILITY OF PACIFIC SALMON SMOLTS TO IHNV

Garth Traxler*, Jon Richard and Kerry Bate

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Nanaimo, BC V9T 6N7

During the 2001-2003 occurrence of IHN in farmed Atlantic salmon in British Columbia, the length of time required for a positive diagnosis was a concern for farmers and fish health professionals. In order to implement various control methods, such as culling or harvesting, which may reduce the farm-to-farm spread of the disease rapid decisions based on accurate tests are required. Atlantic salmon smolts exposed to IHN virus by cohabitation and immersion challenge were sampled daily for a period of 14 days post challenge. Kidney and mucus samples were tested for the presence of virus by cell culture and rt-pcr assays. Virus was first detected on day 3 post-exposure in 1 of 5 fish challenged by immersion by both cell culture and rt-pcr. Over the 14-day monitoring period cell culture resulted in the detection of more positive fish than rt-pcr.

In British Columbia, IHN virus is primarily associated with wild sockeye salmon with the occasional introduction of the virus to farmed Atlantic salmon. The latest occurrence in farmed fish was geographically widespread with 36 net-pen sites affected during the two year period. To address concerns of possible spread of pathogens from farmed fish to wild fish, laboratory challenges of Pacific salmon smolts were conducted to determine their susceptibility. Challenges were conducted in seawater by injection and immersion. These challenge methods were used in order to accurately quantify virus exposure and in the case of intraperitoneal injection, to replicate a worse case scenario by bypassing the fishes first line of defence. Exposed fish were sampled and tested by cell culture for viable virus for 12-days post challenge. Cumulative mortality was monitored in duplicate tanks and all surviving fish were tested for the presence of IHN virus. At the size and age tested all species were quite resistant with sockeye salmon being most susceptible followed by chum salmon. Coho and pink salmon were refractory to IHN virus. These studies provide evidence that transfer of IHN virus from farmed fish to Pacific salmon smolts via waterborne transmission is unlikely.

GENETIC ANALYSIS OF COMPLETE GENES AND GENOMES OF NORTH AMERICAN INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS

Bill Batts*, Gael Kurath, and Jim Winton

Western Fisheries Research Center, USGS, 6505 NE 65th St, Seattle, WA 98115

Infectious hematopoietic necrosis virus (IHNV) infects salmonid fish in western North America, Europe, and Asia. Nucleotide sequences for full length glycoprotein (G) genes previously submitted into the Genbank database are skewed in favor of representing more European than North American strains. Mid-G and partial nucleoprotein (N) sequences have resulted in several publications in which phylogenetic trees have been used to make inferences about patterns of IHNV epidemiology and evolution. These previous studies have resolved over 500 isolates into three major genogroups (U, M, and L) by mid-G sequencing. For this study of complete G & N genes, we selected 26 IHNV isolates (9 U, 9 M, 6 L, and 2 oddballs) and amplified each virus by reverse-transcription nested PCR. Viruses used in this study were isolated from a variety of host species and life stages, and were both temporally (1966-2002) and geographically separated (from California to Alaska, inland to Idaho). We analyzed the nucleotide and deduced amino acid sequences of the full length N and G genes. These sequences can be used to generate more balanced phylogenies to understand additional isolates in the future, and they also allow us to more critically assess the utility of strain typing with mid-G and 5'N sequences.

In addition, we have completed genome sequences for representative IHNV isolates from the U and L genogroups for comparison with the currently available genomes of the WRAC and 32-87, which are both in the M genogroup. In evaluating the open reading frames (ORF) of the large polymerase (L) gene we also observed a larger than usual open reading frame (126-167 amino acids) that potentially may encode a small basic protein (126-165 aa, pI 12.9). Interestingly, this ORF was not seen in the L gene of other closely related fish rhabdoviruses.

MICROARRAY ANALYSIS OF HOMOZYGOUS RAINBOW TROUT FOLLOWING DNA VACCINATION AGAINST IHNV

Maureen K. Purcell[1,2*], Krista M. Nichols[3], Linda K. Park[3], Gael Kurath[2], Gary H. Thorgaard[4], Paul Wheeler[4] and James R. Winton[2]

[1]University of Washington, Seattle, WA, USA

[2]Western Fisheries Research Center/USGS, Seattle, WA, USA

[3]Northwest Fisheries Science Center/NOAA, Seattle, WA, USA

[4]Washington State University, Pullman, WA, USA

*Presenting Author

A highly efficacious experimental DNA vaccine has been developed against the fish rhabdovirus infectious hematopoietic necrosis virus (IHNV) by cloning the viral glycoprotein (G) gene into an expression vector. Plasmid DNA from this vaccine construct induces two phases of protection: an early but transient nonspecific antiviral phase and long-term specific immunity to IHNV. The early anti-viral phase cross-protects against unrelated viruses and has been previously correlated with increased transcription of type I interferon (IFN) stimulated genes. In this study, we examined global gene expression changes during the early anti-viral phase using a 16,000 feature Atlantic salmon microarray developed by the Genomic Research on Atlantic Salmon Project (GRASP; web.uvic.ca/cbr/grasp). Homozygous rainbow trout (Hot Creek strain) were injected intra-muscularly (I.M.) with buffer (n=3), vector DNA (n=3) or the IHNV DNA vaccine (n=4). Fish were sampled at 7 days post-vaccination and RNA from the I.M. site was extracted and evaluated using the microarray. Gene expression of 80 transcripts was significantly modulated in the vector DNA group while over 900 transcripts were changed in the IHNV DNA vaccinated group. Expression of a number of host gene categories was up-regulated in the vector DNA group including a number of genes associated with inflammation, stress and antigen presenting cells (APCs) including a MHC class II gene; these same genes also had increased expression in the DNA vaccinate group. Many more functional gene categories were up-regulated in the vaccinate group, including over 30 genes previously identified as anti-viral or IFN-induced, including key components of the MHC class I antigen presentation pathway. A number of genes were also down-regulated in the DNA vaccinate group (n=281); these genes were primarily related to normal metabolism or muscle structure and function. This study, in combination with others, suggests that the IHNV viral glycoprotein functions as a PAMP (pathogen-associated molecular pattern) capable of stimulating aspects of the rainbow trout innate immune system. A large number of unknown and potentially novel genes which may function in innate immunity were identified during this study. Additionally, the research presented here emphasizes the usefulness of microarray analysis for examining the host-pathogen relationship.

TRANSCRIPTIONAL REGULATION OF TAPASIN, A VIRALLY INDUCED MHC CLASS I PATHWAY MEMBER

Eric D. Landis[1,2*] and John D. Hansen[2]

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All vertebrates, with the exception of bony fish, possess a single, contiguous major histocompatibility complex (MHC). The mammalian MHC contains the vast majority of genes involved in antigen presentation as well as genes important for inflammation. In contrast, the MHC of bony fish is divided into at least four distinct major histocompatiblity "regions". The significance of the split MHC arrangement and its implications for gene function and regulation are unknown. Our lab has been working to better define the contents and arrangement of the MH genomic regions in rainbow trout to learn more about the regulation of antigen presentation in salmonid fish.

The MHC class IA genes are involved in the presentation of viral antigens to cytotoxic T lymphocytes. Owing to their central importance, we have been actively characterizing the regulation of the MHC class I pathway members during IHNV infection. The single class IA gene and associated pathway members are encoded by rainbow trout chromosome 18. Many of the genes in this pathway have been shown to be upregulated in response to acute viral infection in a manner that is reflective of the interferon (IFN) signaling cascade. We have cloned the promoter region of a MHC class I associated gene, Tapasin (TAPBP), which contains binding motifs consistent with IFN regulation. Furthermore, we conducted luciferase reporter assays in a salmonid cell line (STE-137) to show that the TAPBP promoter is responsive to in vitro expression of the rainbow trout interferon regulatory factor-1 (IRF-1) and that this responsiveness is due to a key promoter sequence called the interferon response element (IRSE). These results lay the foundation for the further development of assays for monitoring IFN-mediated responses that are important for fish health.

INACTIVATED IHN VIRUS ELICITS AN EARLY ANTIVIRAL RESPONSE

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The IHN virus DNA vaccine pIHNwG encoding the viral glycoprotein elicits at least three discernable phases of immunity in rainbow trout: (1) an early antiviral response (EVR), (2) a specific antiviral response (SVR) and (3) a late antiviral response (LVR). Whether these stages of immunity are unique to the novirhabdovirus glycoprotein-DNA vaccine type is unclear. To address this question we examined whether administration of exogenous antigen in the form of a whole killed IHN virus vaccine could elicit an early antiviral immune response in rainbow trout similar to that elicited by pIHNwG. The killed IHN vaccines were prepared by inactivation of IHN virus with formaldehyde, binary ethylenimine or β-propiolactone. Rainbow trout immunized by intraperitoneal injection with β-propiolactone inactivated IHN virus elicited a potent early antiviral response 105 degree days after vaccination with relative percent survival (RPS) values between 80-100%. The formaldehyde and BEI inactivated IHN virus vaccines also elicited an early antiviral response in immunized rainbow trout though the RPS values were 50-80%. These results indicate that vaccination of rainbow trout with exogenous IHN virus antigen(s) can elicit an early antiviral response comparable to that elicited by the DNA vaccine pIHNwG and that the inactivating agent used to prepare the antigen can alter the efficacy of the response.

ECOLOGY OF THE SALMONID PARASITE *Ceratomyxa shasta* IN THE KLAMATH RIVER BASIN

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Ceratomyxa shasta has been implicated as a significant source of salmonid mortality in the Lower Klamath River. A study on the prevalence of *C. shasta* infections and the distribution of the parasite's invertebrate host, *Manayunkia speciosa*, was conducted with the objective of determining parasite distribution and abundance in relation to both the salmonid and the invertebrate host. To determine parasite spatial and temporal distribution, sentinel fish were held for 4d at 13 main-stem river locations between Beaver Creek and Keno Reservoir in April, June, July, September and early November 2003. In June 2004, exposures were conducted for 4d at 18 locations between Upper Klamath Lake and the mouth of the Klamath River including several spawning tributaries. Mortality due to infection for groups exposed in the Upper Klamath Basin (< 2.0%) was reduced and delayed compared to mortality in groups exposed in the Lower Klamath basin (>98%). The extreme differences of infection severity observed between groups exposed in the Upper and Lower Klamath Basin suggests that infectious dose is the most likely cause. Studies were also conducted to determine polychaete distribution and habitat ecology. Analysis of benthic samples suggests the polychaete occurs as highly aggregated populations scattered throughout the main-stem river. The polychaete appears to be most significantly influenced by in-stream primary productivity, flow, substrate embeddedness and/or the presence of compact algal epiphytes such as cladophora.

APPLICATION OF A REAL-TIME PCR ASSAY TO DETECT THE MYXOZOAN PARASITE *CERATOMYXA SHASTA* IN ENVIRONMENTAL WATER SAMPLES

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Ceratomyxa shasta is a virulent pathogen of salmonid fish endemic in the Pacific Northwest of North America. Its distribution in the Klamath River, Oregon/California, has been documented using sentinel fish exposures. However, these studies are laborious, time-consuming, use live fish and do not provide data on parasite abundance. Thus, a real-time (or quantitative) polymerase chain reaction (QPCR) assay using TaqMan chemistry was developed to detect the parasite in river water. The assay targeted a 71bp amplicon of the 18S rDNA gene. Standard curves were constructed for known starting numbers of whole spores and for serial dilutions. The assay was sensitive enough to detect $1/1000^{th}$ of a spore, indicating that each had greater than 1000 copies of the target gene. The assay was specific for *C. shasta* and did not detect related parasites, including *Parvicapsula minibicornis*, *Myxobolus cerebralis*, *M. insidiosus* or *Henneguya salmonicola*. Comparison of spiked river water and distilled water samples indicated that some river samples inhibited the reaction but this could be overcome by reducing the sample volume and by including bovine serum albumin in the reaction; occasionally, a second purification step was required. The QPCR methodology was utilised as part of a collaborative project to investigate the temporal and spatial distribution of *C. shasta* in the Klamath River. The parasite was detected throughout the river and several sites of high infectivity were identified where parasite abundance was in excess of 25 spores/L. Two of five tributaries tested contributed parasites to the mainstem river. The QPCR data corroborated results of previous and concurrent sentinel fish exposures. The water sampling and filtering protocol combined with the QPCR assay proved to be a feasible method to detect and quantify parasite levels in environmental water samples. This assay is currently being validated for field use with the objectives of detecting the immediate effects of river flow manipulations and monitoring the long-term effects of hydropower practices.

FISH AT-RISK? A RISK ASSESMENT FOR THE INTRODUCTION AND ESTABLISHMENT OF *MYXOBOLUS CEREBRALIS* INTO THE WILLAMETTE RIVER, OR AND THE STATE OF ALASKA.

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This study examines the risk of introduction and establishment of *Myxobolus cerebralis*, the cause of salmonid whirling disease, into two uniquely different systems: the state of Alaska and the Willamette River, Oregon. Such qualitative risk assessments help decision-makers to eliminate non-issues relatively easily, using logical scientific arguments. These models also help to distinguish critical information gaps and to identify areas for further research concentration.

Alaska

The parasite is not known to occur in Alaska and routes of introduction are suspected to be limited. Also limited are: monitoring or testing for the parasite, and information regarding parasite establishment if introduction were to occur.

Critical data gaps identified in the release assessment:

- Long-distance straying of fish from endemic areas
- Infected migrants in commercial harvests & disposal methods for effluent and carcasses.

Critical data gaps identified in the exposure assessment:

- *T. tubifex* populations & their spatial/ temporal overlap with fish hosts.
- Worm lineages: Preliminary studies show existence of lineage IV, never before described in North America. We are examining the susceptibility of this lineage to *M. cerebralis*.

Willamette River, Oregon

There are numerous potential introduction routes throughout the Columbia River Basin (straying of anadromous fish from endemic areas, angler activity, transfers of infected fish between private ponds) though environmental conditions and the low abundance of tubificid hosts may limit the parasite's establishment in certain areas.

Critical data gaps identified in the release assessment:

- Stray fish: Our studies have been the first to document significant numbers of stray steelhead in the Willamette River. Data being gathered from strays includes: their system or hatchery of origin, and potential parasite infection.
- Private ponds/hatcheries and their history of *M. cerebralis* infection.

Critical data gaps identified in the exposure assessment:

- *T. tubifex* populations, distributions, and lineages.

As these information gaps narrow, managers and scientists will gain a better understanding of where to allocate resources to help prevent further spread and effects of the disease.

SUSCEPTIBILITY OF RIO GRANDE CUTTHROAT TROUT (*Oncorhynchus clarki virginalis*) TO EXPERIMENTALLY INDUCED INFECTION WITH *Myxobolus cerebralis*

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Whirling disease is a parasitic infestation of cartilage in salmonids by the myxosporean *Myxobolus cerebralis*. We evaluated the susceptibility of Rio Grande cutthroat trout (RGCT; *Oncorhynchus clarki virginalis*) to infection by *M. cerebralis* through paired laboratory challenges using rainbow trout (RBT; *O. mykiss*) of known sensitivity to whirling disease. This research not only characterizes disease susceptibility in RGCT, but also provides a standardized assessment to compare disease susceptibility with other native and inland cutthroat trout species. The treatments were parasite concentrations (0, 50, 100, 250, 500, 1000 triactinomyxons/fish) within a replicated complete block design using fry at 600 degree-days. The laboratory challenge was terminated at 130 days post-exposure (1300 degree-days). Diagnostic metrics included survival, clinical symptoms (behavioral, coloration, and skeletal abnormalities), histology for *M. cerebralis*, and swimming stamina challenge. Clinical symptoms of whirling disease (whirling behavior and black-tail) were observed within both species at the 500 and 1000 triactinomyxons/fish dosage. Rio Grande cutthroat trout, however, exhibited significantly reduced survival and a pronounced parasite concentration response when compared to RBT. Rio Grande cutthroat trout exhibited significantly lower swimming speeds compared to RBT, but these were not related to parasite concentration. In contrast, RBT exhibited significantly lower swimming speeds with increasing parasite concentration. Preliminary histology scores indicate there were no significant differences in disease severity between RBT and RGCT subjected to the stamina challenge. A significant negative parasite concentration response, however, was exhibited by both RBT and RGCT. Preliminary results indicate that if *M. cerebralis* were to spread to waters supporting RGCT, the species would be at great risk of infection and could experience significant population declines.

COMBINING SUPPRESSION SUBTRACTIVE HYBRIDIZATION AND MICROARRAYS TO MAP THE INTRA-SPECIFIC PHYLOGENY OF *Flavobacterium psychrophilum*

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Flavobacterium psychrophilum is the etiological agent of bacterial coldwater disease, which causes significant problems to aquaculture worldwide. To determine the relatedness of multiple isolates, reciprocal subtractive libraries were prepared for virulent (CSF 259-93) and avirulent (ATCC 49418) strains, and the distribution of unique sequences among 34 strains spanning three continents was assessed using DNA microarrays. This analysis divided *F. psychrophilum* into two major lineages (I and II). During this study we identified two 16S rRNA sequence variants (6 base differences) for *F. psychrophilum* strains ATCC 49418 and CSF 259-93 and consequently augmented an existing 16S rRNA microarray to detect both sequence variants. Microarray experiments showed that CSF 259-93 hybridized as expected, but ATCC 49418 was positive for both sequence variants. We then developed a PCR-RFLP assay (*Mnl*I and *Mae*III) to distinguish between the two sequences. Gel isolation of PCR-RFLP products, cloning, and sequencing confirmed that ATCC 49418 harbors both 16S rRNA sequences. Microarray experiments showed that 11 of 14 strains from genetic Lineage I harbor both the CSF 259-93 and ATCC 49418 16S rRNA sequence variants, whereas another 15 of 15 Lineage II strains were only positive for the CSF 259-93 sequence ($P < 0.0001$). Elastin hydrolysis and tetracycline resistance were most closely associated with the latter strains ($P < 0.0001$ and $P = 0.024$, respectively). These data collectively support the hypothesis that *F. psychrophilum* is composed of at least two distinct genetic lineages that are closely associated with host species origin.

DEVELOPMENT OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR DETECTION OF *Flavobacterium psychrophilum*

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Flavobacterium psychrophilum, the etiologic agent of bacterial coldwater disease (BCWD), is a significant bacterial pathogen in salmonid aquaculture. Despite widespread interest in BCWD, commercial vaccines have not been developed. Antimicrobial treatment options are limited in the United States and these options are further limited by increasing resistance among isolates of *F. psychrophilum*. Vertical transmission of the pathogen appears to occur, thus the objective of the present study is to develop a quantitative method to screen broodstock kidney homogenate and ovarian fluid for the presence of *F. psychrophilum*. At present we have four mouse hybridoma clones producing monoclonal antibodies against *F. psychrophilum* (CSF 259-93) outer membrane epitopes. Monoclonal antibody FL-43 is reactive with *F. psychrophilum* CSF 259-93 (virulent strain) and *F. psychrophilum* ATCC 49418 (avirulent strain), but not with *Flavobacterium columnare*, whereas FL-15 is reactive with both *F. psychrophilum* and *F. columnare*. FL-43 is being used as a capture antibody and FL-15 as a detection antibody for a double-antibody sandwich ELISA. These antibodies appear to be suitable for sensitive detection and quantification of *F. psychrophilum* in infected tissue homogenate. Results on the optimization experiments and potential application of this assay for screening infected broodstock will be discussed.

EVALUATION OF LETHAL AND NON-LETHAL SAMPLING FOR THE DETECTION OF WSIV INFECTION IN KOOTENAI RIVER WHITE STURGEON (Acipenser transmontanus)

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The Kootenai Tribe of Idaho White Sturgeon Conservation Hatchery (KTOI) implements an annual histological examination to screen for the presence of white sturgeon iridovirus (WSIV) infection in juvenile sturgeon prior to their release into the Kootenai River. Presumptive diagnosis for the presence of infection relies on lethal sampling to identify hypertrophied infected cells within epithelial tissue of the skin, gills, oropharynx, and olfactory (barbels) organs. In the present study, non-lethal sampling and PCR of fin punch biopsy tissue were compared to the standard lethal sampling procedure for the ability to detect viral infection in asymptomatic carriers and sturgeon undergoing a WSIV disease outbreak. To initiate infection, duplicate groups of 100 juvenile sturgeon (mean wt. 2.0g) were co-habitated with either 10 fish that were immersion challenged with WSIV or mock immersion challenged with MEM-10. Five fish from each tank were randomly sampled weekly for 8 weeks. The head of each fish as well as left pectoral fin tissue, extracted using a hand held paper-hole punch, were placed in 10% neutral buffered formalin and processed for histological examination. A portion of the right pectoral fin was removed using a scalpel for PCR testing. An identical sampling procedure was performed on sub samples of 25 sturgeon (mean wt. 3.5 g) from three rearing tanks at the KTOI hatchery. These tanks consisted of sturgeon surviving a WSIV disease outbreak, siblings showing no signs of infection, and a different group of fish with no known WSIV history. Results of this study are currently being analyzed and will be discussed.

THE LIFE CYCLE OF *PARVICAPSULA MINIBICORNIS*, A MYXOZOAN PARASITE OF SALMONIDS, INVOLVES THE FRESHWATER POLYCHAETE *MANAYUNKIA SPECIOSA*

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In the Klamath River, Oregon/California, USA, severe infections by the myxozoans *Parvicapsula minibicornis* and *Ceratomyxa shasta* have been documented in seaward-migrating juvenile Chinook salmon in recent years. The invertebrate host for *C. shasta*, the freshwater polychaete *Manayunkia speciosa*, is known to occur in high densities in specific habitats throughout the river. The similar distribution pattern of *P. minibicornis* and *C. shasta* infected fish suggested that the polychaete may serve as host for both of these myxozoans. We recently confirmed this hypothesis by amplification of *P. minibicornis* 18S rDNA from polychaetes infected with a small (5-8 μm) spherical actinospore. The actinospore was distinguished by having three spherical polar capsules that protruded from the apical end and no processes. Actinosporean developmental stages were observed in the coelom of the polychaete, rather than in the body wall as described for *C. shasta*. DNA from spores was amplified, sequenced, and determined to be at least 99.6% similar to *P. minibicornis* myxospores from British Columbia, Canada (GenBank #AF201375), which verified the proposed life cycle. Chinook salmon were exposed to 1,000 actinospores per fish to demonstrate infection of the vertebrate host. Prevalence of infection in polychaetes collected from one site in the Klamath River during the period of peak salmon migration was 5% for each of *P. minibicornis* and *C. shasta*.

POSTER SESSION

EFFECTS OF TEMPERATURE AND DESICCATION ON *Ceratomyxa shasta* ACTINOSPORE PRODUCTION AND RELEASE FROM THE POLYCHAETE HOST *Manayunkia Speciosa*.

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Ceratomyxa shasta, the cause of Ceratomyxosis, has a life cycle requiring both a salmonid and polycheate host. Thus far, little is known about the effects of environmental variables on parasite replication in the polychaete. To test the effects of temperature, algae and sediment containing *M. speciosa* was collected from the Klamath River where *C. shasta* is endemic, and maintained in the lab at four temperatures ranging between 5º C and 25º C. In addition, tolerance of desiccation was tested by drying the material for 24 hours and then rehydrating and maintaining it at 12º C. Prior to treatment, polychaete density was determined for the population. To measure rates of parasite release, 1 L water samples were collected from each of the treatments twice weekly and suspended material was collected on filters for assay by quantitative PCR. At termination of the experiment, final polychaete density was determined and PCR used to establish the percent of infected worms.

PREVALENCE OF *Renibacterium salmoninarum* INFECTION AMONG JUVENILE CHINOOK SALMON IN NORTH PUGET SOUND AND IMPLICATIONS FOR DISEASE INTERACTIONS

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Renibacterium salmoninarium causes bacterial kidney disease (BKD), a chronic and sometimes fatal condition of salmon and trout. This bacterium is transmitted both horizontally and vertically, and high prevalence in a population can lower overall fitness. The purpose of this study was to determine infection prevalences of *R. salmonarium* among nearshore juvenile chinook salmon in North Puget Sound. The central questions were whether the prevalence rates would vary by fish origin (marked or hatchery vs. unmarked or feral), by season, or by geographical area. Juvenile chinook salmon were collected between April and October in 2002 and 2003 in 32 nearshore habitat sites by surface trawl (townet). Kidney tissue samples were collected and analyzed microscopically by counting bacterial cells stained with an anti-*R. salmonarium* polyclonal antibody conjugated to fluorescein isothiocyanate. Differences in infection prevalence were observed by geographical area and by season. Although seasonal differences were observed among the distinct geographical areas, no overall seasonal trend was found throughout the North Puget Sound. There was no difference in infection prevalence between marked and unmarked fish in all regions in the North Puget Sound. Our findings suggest potential disease interactions, possibly through horizontal transmission, between the feral and hatchery fish.

DETECTION OF *Myxidium* spp. IN PET SHOP GOLDFISH

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Many fish species have been dispersed throughout the world through the aquarium industry. A number of these have been accidentally and or intentionally released into the wild and subsequently proliferated; one such species is goldfish (*Carassius auratus*). To look at the potential for introducing myxozoan parasites by this pathway, we purchased 2 groups of goldfish from a national aquarium store chain. Both groups consisted of 10 goldfish ranging in size from 2 – 10 cm. A complete necropsy was performed on each fish and any myxospores detected by light microscopy were photographed and categorized by key to genus level. A heavy infection of *Myxidium* spp. was observed in the gall bladders of 14 of the 24 goldfish. To support the phenotypic description of the *Myxidium*, the DNA was extracted and the 18S rDNA gene was amplified using primers specific for the Myxozoan group. The amplified product was sequenced and compared to those available in the GenBank database.

MULTIFARIOUS MYXOZOANS: SPORE HUNTING IN OREGON

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An excellent opportunity for expanding our knowledge of myxozoan parasites was provided through coordination with sampling efforts to survey and manage the prevalence of known myxozoan fish pathogens in the Pacific Northwest, chiefly *Myxobolus cerebralis* and *Ceratomyxa shasta*. Most myxozoans are believed to have a two-host lifecycle, developing as a myxosporean in fish and an actinosporean in annelid worms, but this has been demonstrated for only 2% of known species. Over 18 months, we examined both wild and sentinel fish as well as samples of stream substrates containing oligochaete and polychaete worms from 6 rivers, their tributaries and several fish hatcheries. A wide range of myxozoans were identified, comprising 45 myxosporean records and 53 actinosporean records. From fish, *Chloromyxum* and *Myxobolus* were the most speciose genera with at least 3 and 7 species respectively. Other myxosporean genera included *Sphaerospora*, *Myxobilatus*, *Henneguya*, *Parvicapsula*, *Zschokkella* and *Myxidium*. A wide range of actinosporean collective groups were recorded from oligochaete and polychaete worms, including Triactinomyxon (10 types), Raabeia (3), Echinactinomyxon (8), Aurantiactinomyxon (3), Siedleckiella (2), Tetractinomyxon (2) and Antonactinomyxon (1). At least 2 novel host oligochaete species were found: *Nais pseudobtusa* and *Spirosperma nikolski*. Sequencing and comparison of 18S rDNA from the fish myxosporean and worm actinosporean stages is underway to determine relationships among these organisms, including if any are alternate lifecycle stages. Using sequencing and infection experiments, we have successfully elucidated the lifecycles of *Chloromyxum auratum* and *Parvicapsula minibicornis*.

CHARACTERIZATION OF THE IDAHO NEUROTROPIC *Myxobolus*, AND IT'S DISTRIBUTION IN IDAHO WATERS

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Abstract

While screening salmonids for *M. cerebralis*, the causative agent of whirling disease, we detected another *Myxobolus* sp., morphologically similar to *M. cerebralis*, in brain tissue. Our initial objective was to develop a PCR-based technique to discriminate between *M. cerebralis* and the neurotropic *Myxobolus*. The resulting diagnostic tool has resolved cases in which the pepsin-trypsin digest (PTD) screening test indicated *Myxobolus* spores but histological examination or PCR was negative for *M. cerebralis*. We then looked at the relatedness of this neurotropic *Myxobolus* to other known *Myxobolus* species. Our phylogenetic analysis based on 683 bp of the 18S ribosomal DNA reveals a large genetic divergence between the Idaho neurotropic *Myxobolus* and *M. cerebralis* suggesting this neurotropic *Myxobolus* is a new, previously undescribed species. Continuing to study this neurotropic Myxobolus we now have 1) characterized the species in preparation for naming it, and 2) using our PCR-based diagnostic technique, mapped it's distribution in Idaho.

ORAL PRESENTATIONS

BIOCHEMICAL AND MOLECULAR TECHNIQUES FOR STRAIN TYPING OF *Mycobacterium marinum*

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Infections by various species of mycobacteria are relatively common in a wide variety of wild and captive fishes. In particular, striped bass and hybrid striped bass (HSB) are especially susceptible to *Mycobacterium marinum* infections. The precise genotypic and phenotypic characterization of *Mycobacterium* spp. can provide important information on the source of infections and useful information for developing control and prevention strategies. To this end we conducted various biochemical and molecular procedures on *M. marinum* isolates collected throughout the United States. These isolates were obtained from HSB and other fishes, including several collected over a five year period from a single HSB farm in California. Using polymerase chain reaction amplification and subsequent sequencing of ITS rDNA from these isolates, we identified two separate groups differing from each other by a single base pair substitution, designated group A and group G. Consistent with these results, biochemical analysis revealed that the group A isolates were positive from Tween hydrolysis, while group G isolates were negative. To determine if these distinct groups were clones, i.e. the same strains, DNA from the isolates was digested with restriction enzymes and analyzed by pulsed field gel electrophoresis (a DNA fingerprinting technique). The group A isolates were determined to consist of a variety of strains, while the group G isolates were clonal. Indeed, *M. marinum* associated with the ongoing infection in the HSB farm in California belonged to group G, and the same strain was found in HSB from a Mississippi farm. Our data demonstrate that this isolate of *M. marinum* is geographically widespread. We have identified biochemical and molecular markers for this strain which can be useful in tracking the source of infection, identifying both reservoir hosts and off host proliferation, and brood stock screening.

THE NATIONAL AQUATIC ANIMAL HEALTH PLAN: BRIEFING AND UPDATE

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The Joint Subcommittee on Aquaculture's National Aquatic Animal Health Task Force has been charged with the mission to develop and implement a national aquatic animal health plan (NAAHP) for aquaculture in partnership and cooperation with industry, regional organizations, State, local, tribal governments and other stakeholders. The purpose of the NAAHP is to foster and support effective and efficient aquaculture; to protect the health of our nation's wild and cultured aquatic resources; and to meet our national and international trade obligations. The NAAHP is being developed via input from a series of Task Force-associated working groups. Each working group consists of 10-20 experts, each representing a sector of the aquaculture community. Each work group focuses on a specific element of the NAAHP such as: roles and responsibilities of health professionals, laboratory methodologies, and species-specific working groups that address issues for that sector. Several work groups have met and recommendations are considered when drafting the NAAHP. The first complete draft of the NAAHP is expected to be completed in the spring of 2007, with refining and implementation to follow. The NAAHP in itself will not be codified into regulation; however, implementation of certain elements, such as import requirements, may require revisions to existing laws, regulations or policies.

BREAKING THE CYCLE: USING COMBINATIONS OF ANTIBIOTICS AND VACCINATION TO PREVENT AND TREAT BACTERIAL KIDNEY DISEASE

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Bacterial kidney disease (BKD) is a continuing problem for salmon culture, in spite of broodstock segregation and antibiotic application. Management practices for BKD can be variable because the etiological agent, *Renibacterium salmoninarum*, is transmitted both vertically and horizontally. We have identified a compound vaccine with both therapeutic and prophylactic value, and we have focused on combinations of vaccination with antibiotic application for juvenile chinook salmon. Our future directions in developing integrated BKD management, including progress on an attenuated *R. salmoninarum* vaccine candidate, will be presented.

RISK CHARACTERIZATION OF ERYTHROMYCIN EFFLUENTS FROM SALMON HATCHERIES

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We characterized the worst-case scenario erythromycin effluents at INAD 6013 participating fish hatcheries in Idaho, Oregon and Washington from 1998 to 2004. The hatcheries included tribal, state agency and federal facilities. The total erythromycin medicated feed use was summarized by hatchery facility from reports of use in lbs of feed used for each treatment. This quantity was converted to kg of erythromycin base in each use. The total was divided by the number of days that rations were fed, and the results expressed as amount per 24 h as effluent based on total hatchery flow rates during the treatment and expressed as g erythromycin base per L. The worst case effluents ranged from a low of 0.003 µg/L to a high of 77 µg/L. Hatcheries in Washington and Oregon had more consistent patterns in average discharge over the 8 years of the study than facilities in Idaho. Up to 30% of facilities in Oregon and Washington had worst case scenario effluents less 1 µg/L, the no effect limit of the Food and Drug Administration. Hatcheries in Idaho averaged higher mean effluent concentration across all years when using only hatchery discharge as a dilution factor. Most facilities in Idaho use settling ponds at all times, and do not discharge directly to the rivers. These factors likely reduce the risks to aquatic flora and fauna near hatchery outflows.

SOMETHING OLD, SOMETHING NEW: MYCOBACTERIOSIS IN SALMON AND ZEBRAFISH.

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Mycobacterial infections in fish are traditionally attributed to strains of *Mycobacterium marinum*, *M. chelonae*, and *M. fortuitum*. Indeed, *M. chelonae* has been isolated several times from salmonid fishes in the Pacific Northwest. In the 1980's, based on biochemical analyses of these isolates, researchers suggested that strains infecting salmon represent a distinct subspecies of *M. chelonae*. However, due to the heterogeneity within the species *M. chelonae*, this assertion has not been widely accepted. Using these same isolates, we revisited this issue using molecular techniques. A DNA fingerprinting method (pulsed field gel electrophoresis) indicated that there are multiple strains of *M. chelonae* associated with infections in salmon. In spite of this overall genetic heterogeneity, DNA sequence analyses of both 16S and *hsp65* DNA sequences revealed that *M. chelonae* isolates from salmon form a phylogenetic grouping to the exclusion of other *M. chelonae* isolates that have been sequenced (including several isolates from other fish species). Thus, our data support the earlier finding that isolates of *M. chelonae* from salmon represent a distinct subspecies of mycobacteria. More recently, mycobacteriosis has been recognized as a significant disease problem in zebrafish (*Danio rerio*), an important model organism for research. Strains of *M. chelonae* are commonly isolated from zebrafish and these are usually associated with incidental disease in research colonies. However, during severe outbreaks, other species of mycobacteria are usually identified. Interestingly, these other species are more commonly associated with diseases in humans, although those from zebrafish may represent fish-specific strains. Monitoring and characterization of mycobacteria from zebrafish is ongoing and potential risks to human health are being explored.

DEVELOPMENT AND DISORDERS OF OPAKAPAKA (*Pristipomoides filamentosus*) LARVAE IN CULTURE

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Opakapaka (*Pristipomoides filamentosus*) is a snapper (Lutjanidae) native to waters around Hawaii. Recent population declines of this commercially important species have resulted in increased interest in culture of this fish. In culture, survival of this fish through the larval period has been consistently low, usually not exceeding 2%. To elucidate the causes of these mortalities, the morphologic development of the digestive, respiratory, and skeletal systems were investigated through examination of histological sections and stained whole fish. It was found that crucial periods of development in both the digestive system and the respiratory system coincided with catastrophic mortality events in larvae. Additionally, lower jaw deformities occurred as early as 2 days post hatch at a prevalence of 20%. Vertebral column deformities first appeared at about 10 days post hatch and were detected in 10**%** of analyzed larvae. Delayed development and deformities likely lead to starvation and consequent larval mortality, as the proper and timely development of these organ systems is essential for larval ability to capture and process food. It is therefore recommended that the exact cause of larval skeletal deformity be determined**,** broodstock nutrition be supplemented during spawning season, and any additional stress to larvae during crucial periods of development be minimized. Understanding the development of opakapaka larvae will assist in identifying fish readiness for food, determining optimal food type, and adjusting environmental conditions to appropriate levels.

DISSEMINATED NEOPLASMS OF EASTERN PACIFIC MUSSELS, *Mytilus trossulus*

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Disseminated neoplasia, also known as hemic neoplasia, is a leukemia-like disease of numerous species of bivalve mollusks worldwide. In Eastern Pacific *Mytilus trossulus*, the disease causes significant mortality in all age classes. Similar if not identical conditions have been reported in *M. trossulus* from other geographic regions throughout the northern hemisphere. Disseminated neoplasms are rare to absent in *M. edulis* and *M. galloprovincialis*, including individuals from locations where the disease is common in *M. trossulus*. Flow cytometric DNA content analyses demonstrated that neoplastic cells in *M. trossulus* from British Columbia, Washington and Oregon have a distinct G_0G_1 DNA level of either tetraploid (4n) or approximately pentaploid (5n). The two forms appear to arise from discrete transformation events that result in independent pathogenetic sequences. One unique feature of this disease within the realm of oncology is that the primary mode of spread between individuals appears to be direct transplantation of intact cells. The neoplastic features of these cells make them excellent candidates for source material to establish the first marine invertebrate cell line.

EVALUATION OF MACROPHAGE AGGREGATES IN SALMONID FISHES

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Macrophage aggregates (MAs) occur in various organs of fishes, especially the kidney, liver, and spleen, contain melanin, ceroid/lipofuscin, and hemosiderin pigments, and have been used as indicators for a number of natural and anthropogenic stressors. Macrophage aggregates occur in salmonids but are poorly organized, irregularly shaped, and are generally smaller than those in derived teleosts. These features complicate quantification, and thus these fishes have seldom been used in studies correlating MAs with environmental stressors. To alleviate these complications, we developed color filtering algorithms for use with the software package ImagePro Plus®, (Media Cybernetics, Silver Spring, MD) that select and quantify pigmented area (i.e., colors ranging from light yellow to brown to black) in tissue sections. Image analysis results compared well with subjective scoring when tested on brook (*Salvelinus fontinalis*) and rainbow (*Oncorhynchus mykiss*) trout captured from high-elevation lakes or hatcheries. Macrophage aggregate pigments correlated positively with age and negatively with condition factor. Within individual fish, pigmentation correlated positively between organs, suggesting that the kidney, liver, or spleen were suitable indicator organs. In age-matched fishes, MA pigments were not different between hatcheries and lakes in organs examined. Between lakes differences in pigments were observed in the kidney and spleen, but were not explained by age, condition factor, sex, or maturation state. Our results indicate that quantification of area occupied by MA pigments is an efficient and accurate means of evaluating MAs in salmonid organs and that organ pigmentation correlates with age and condition factor, as seen in studies with other fishes.

DEVELOPMENT OF MARKERS FOR T LYMPHOCYTES IN SALMONID FISH: GENERATION OF SPECIFIC MONOCLONAL ANTIBODIES AGAINST RAINBOW TROUT LCK

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A lack of suitable reagents has impeded the study of T lymphocytes and T-cell mediated immunity in salmonid fish. While genes for molecules typically involved in T-cell receptor signaling have recently been identified, the ability to isolate and identify specific lymphocyte populations has not been possible. Thus it is important to develop reagents specific for salmonid T lymphocytes to facilitate progress in this area.

Lymphocyte specific kinase (LCK) is an intracellular molecule that is necessary for signal transduction in mammalian T-cells. Following antigen presentation by MHC, LCK activates molecules associated with the T-cell receptor complex (e.g. CD3 and TCR-ζ) and the kinase ZAP-70 to initiate T-cell activation and proliferation. We have isolated two copies of LCK from rainbow trout that are abundantly expressed in thymus and appear to show differential regulation in peripheral blood lymphocytes in response to T-cell mitogens PHA and PMA. Salmonid LCK is highly conserved and possesses a conserved CXXC motif in the N-terminal (unique) domain that in mammals associates with the cytoplasmic tails of CD4 and CD8 molecules on helper and cytotoxic T-cells. Recombinant LCK1 was used to immunize rats for the generation of monoclonal antibodies (mAbs). Characterization of these mAbs identified one mAb (30A8) that is strongly specific for trout LCK. 30A8 detects two distinct protein bands in trout thymus lysates, immunoprecipitates a native 56 kDa protein from thymus lysates, and is suitable for intracellular staining of LCK containing lymphocytes using FACS analysis. Coupled with other reagents that are being developed within our laboratory (CD8α and CD4 molecules), this mAb will be important for progressing our knowledge of T-cell activity and cell-mediated immunity in salmonids.

DOES THE SALMON LOUSE *Lepeophtheirus salmonis krøyer* OCCUR ON THREESPINE STICKLEBACK (*Gasterosteus aculeatus* L.) IN COASTAL BRITISH COLUMBIA?

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The salmon louse *Lepeophtheirus salmonis* is a caligid copepod parasitic on anadromous salmonids in the marine environment and has a circumpolar distribution in the Northern Hemisphere. The parasite has infrequently been reported on non-salmonid hosts. The epizootiology of *L. salmonis* and another caligid copepod, *Caligus clemensi* has been studied on juvenile Pacific salmon for three years in a coastal ecosystem in British Columbia (B.C.). Sea lice were frequently observed on threespine stickleback (*Gasterosteus aculeatus*) caught as by-catch in this study. This report documents the prevalence and abundance of sea lice on stickleback in 2003 and 2004 and describes *L. salmonis* for the first time as a parasite of *G. aculeatus*. Fish were individually bagged directly from beach or purse seines and immediately stored at -20ºC for microscopic examination. Each fish was weighed and measured and sea lice were counted and identified to species and stage. A total of 4,225 sticklebacks were examined: 2,815 in 2003 and 1,410 in 2004. In 2003, 61.3% of sticklebacks were infected with 10,272 sea lice. The prevalence and abundance of motile *C. clemensi* was 11.5% and 0.18 lice per fish. The prevalence and abundance of a motile *Lepeophtheirus* sp. was 1.4% and 0.02. The prevalence and abundance of all non-motile (chalimus and copepodid) stages was 60.7% and 3.46, respectively. Of 2,872 non-motile stages identified to genus, 93.6% were *Caligus* and 6.4% *Lepeophtheirus*. In 2004, 23,691 sea lice were collected from 83.3% of sticklebacks and both species of sea lice were again found. *Lepeophtheirus* sp. (prevalence, 82.3%; abundance, 14.9) was more frequently observed than *C. clemensi* (prevalence, 42.3%; abundance, 1.8). The most abundant stage of *Lepeophtheirus* was the copepodid (40.2%) whereas preadult and adults accounted for 2.4% of all stages. Motile specimens were identified as *L. salmonis* by using morphological criteria. The most abundant stage of *C. clemensi* was the chalimus I (37.2%) whereas preadults and adults accounted for 3.8% (n=99) of all stages. Nucleotide sequence data obtained from the 18SrRNA and Cytochrome C Oxidase I genes of several *Lepeophtheirus* spp. and *C. clemensi* supported the conclusion that the parasite from sticklebacks was *L. salmonis*.

Thus sea lice were considerably more abundant on sticklebacks in 2004 than in 2003 and the proportion of *Lepeophtheirus* was greater in 2004. These patterns of infection are similar to those observed on juvenile chum and pink salmon inhabiting the same coastal ecosystem. The presence of adult *L. salmonis* indicated that the threespine stickleback is a reservoir of infection in the region. There are no reliable data on the abundance of *G. aculeatus* in coastal B.C so the magnitude of its role as a reservoir is uncertain. In addition, stickleback may influence the epizootiology of *L. salmonis* by competitively interfering with transmission of this parasite to salmonids.

SURVIVAL OF NEW ZEALAND MUDSNAILS (*Potamopyrgus antipodarum*) IN THE GASTROINTESTINAL TRACT OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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Since their recognized introduction to the United States in 1987, the New Zealand mudsnail (*Potamopyrgus antipodarum*) has been found in many locations throughout the western United States. This invasive species has become established around and in several fish hatcheries. In high biomass, the New Zealand mudsnails can affect functioning of the aquatic ecosystem. Fish stocking and transfers from hatcheries may accelerate the spread and introduction of the snails to other locations, as snails have been reported to survive passage through the gastrointestinal tract of trout. We are examining the survival and passage of New Zealand mudsnails in the gastrointestinal tract of rainbow trout (*Oncorhynchus mykiss*). We have conducted several laboratory trials by feeding snails to individual trout, held in four aquaria. Fish were sampled at four time intervals following feeding to determine the number of snails alive and dead in the stomach, anterior intestine, and posterior intestine. We found live snails in the stomach, but snails in the anterior intestine and posterior intestine were dead. We are evaluating the effects of fish, snail size, and fish feeding regimes on the survival of ingested snails. This research will pose a potential depuration strategy to reduce the risk of transferring snails during fish stocking.

MICROBIAL PROFILES FROM RAINBOW TROUT INTESTINES, NEW ZEALAND MUDSNAILS, AND HATCHERY INFLOW AND OUTFLOW AT HAGERMAN STATE FISH HATCHERY, IDAHO

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We characterized the aerobic heterotrophic bacteria of the posterior intestines of hatchery rainbow trout, the water from the raceway inflow and outflow, the fish feed, and New Zealand mudsnails residing in the raceway intake and outflow at Hagerman State Fish Hatchery during December 2005. We calculated the colony forming units for each on tryptic soy agar, described the isolates, and selected isolates for characterization by API methods. We selected isolates to test for sensitivity to oxytetracycline and chlorophenicol. Fewer than 10% of all isolates were resistant to oxytetracycline, including an isolate identified as *Streptococcus bovis* that was isolated in the raceway effluent water. Total densities per mL or g recorded as colony forming units (CFU) of bacteria and yeasts were 4-5 X higher in effluent water than in inflow water, and nearly 20 times higher in snails in the effluent than in snails tested from the areas of inflow. The CFU/g of the posterior intestine ranged from 2 to 500 X 10^5. The implications of bacterial concentration in New Zealand mudsnails are addressed.

CHANGES IN HEMATOLOGY IN ATLANTIC SALMON AFTER EXPERIMENTAL CHALLENGE WITH IHN VIRUS: PRELIMINARY RESULTS

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Two groups of 75 juvenile Atlantic salmon were experimentally infected with IHN virus, one via a waterborne challenge and the second by cohabitation with experimentally infected cohorts. Five fish from each group were randomly selected and humanely sacrificed each day for 14 days post challenge. Peripheral blood smears and impression smears of kidney and spleen were made from each fish. Changes in haematopoietic cell morphologies were scored. Preliminary results on the comparison of experimentally challenged and control groups will be presented.

BCSFA FISH HEALTH DATABASE – RETROSPECTIVE OVERVIEW

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The British Columbia Salmon Farmers Association's Fish Health Database is a public-private partnership. Since the end of 2002 salmon aquaculture sites in BC have entered their fish health information into the system on a monthly basis, as a condition of license. The data is reported quarterly on an aggregate basis to the BC Ministry of Agriculture, Food and Fisheries (BC MAFF) and is subject to audit by BC MAFF. Summarized data from the first two years of operation will be presented.